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54 LH-RH antagonists.

57 Disclosed herein are peptide analogs of the luteinizing hormone releasing hormone (LH-RH) which are very potent antagonists of LHRH. These analogs are even active when administered through the oral route.

They differ in structure from LH-RH by having other amino acid residues at the positions 1 and 2, by the presence of a basic amino acid residue at position 6 and by the optional presence of other amino acid residues at the positions 3 and 10.

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LH-RH ANTAGONISTS

This invention relates to novel peptides which are antagonist of the luteinizing hormone releasing hormone (LH-RH), which has the structure:

p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>.

5 More specifically, this invention relates to luteinizing hormone releasing factor (LH-RH) analogs, salts thereof, to processes and intermediates for preparing these analogs, and to pharmaceutical compositions pertaining to these  
10 analogs.

The LH-RH analogs of this invention differ in structure from LH-RH by having the amino acid residues at positions 1, 2 and 6, and optionally at positions 3 and 10, replaced by other amino  
15 acid residues.

For several years investigators have been searching for selective, potent antagonists of the LH-RH decapeptide. See the review article by  
25 D.H. Coy and A.V. Schally, Annals of Clinical Research, 10, 139 (1978). The high degree of interest in such antagonists is due to their usefulness in the endocrine and cancer fields.

A great number of compounds have been prepared as potential LH-RH antagonist but most of these compounds lack potency or are mixed agonist and antagonist of the LH-RH decapeptide. The most  
5 interesting antagonists to date have been compounds having a modified structure of LH-RH. For instance, [D-Phe<sup>2</sup>]-LH-RH, R.A.W. Rees et al., J.Med.Chem., 17, 1016 (1974); [D-Phe<sup>2</sup>,D-Phe<sup>6</sup>]-LH-RH, [D-Phe<sup>2</sup>,Phe<sup>3</sup>,D-Phe<sup>6</sup>]-LH-RH and [D-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Phe<sup>6</sup>]-LH-RH,  
10 D.H. Coy et al., in "Peptides 1976", A. Loffet, Ed., Editions de l'Université de Bruxelles, Brussels, Belgium, 1977, p. 463; [D-p-F-Phe<sup>2</sup>-D-Ala<sup>6</sup>]-LH-RH, C.W. Beattie et al., J.Med.Chem., 18, 1247 (1975) and [Ac-D-Phe<sup>1</sup>,D-Phe<sup>2</sup>,D-Trp<sup>3,6</sup>]-LH-RH,  
15 K. Channabasavaiah and J.M. Stewart, Biochem. Biophys.Res.Comm., 86, 1266 (1979) and the peptides disclosed in the U.S. patent specification No. 4,317,815.

20 The present invention provides pure LH-RH antagonists that are more potent than any of the LH-RH antagonists reported to date; and moreover exert their activity also when administered through the oral route.

25 The compounds of this invention, i.e. the LH-RH analogs, are represented by formula I  
$$X-R^1-R^2-R^3-Ser-Tyr-R^4-Leu-Arg-Pro-R^5-NH_2 \quad (I)$$
  
in which X is hydrogen, lower alkanoyl, HOOC-(CH<sub>2</sub>)<sub>n</sub>-CO wherein n is an integer from 2 to 6, or the  
30 acyl or N-alkanoyl-acyl portion of glycine or a D- or L-amino acid; R<sup>1</sup> is Gly, L-Ala, L-3-(1-naphtyl)-Ala, L-3-(2-naphtyl)-Ala, D-Ala, D-3-(1-naphtyl)-Ala, D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-Phe having one or more substituents at the  
35 phenyl moiety selected from halogen, nitro, amino,

alkyl (1-4 C), cyano, trifluoromethyl, hydroxy and alkoxy (1-4 C);  $R_2$  is D-Phe having one or two substituents at the phenyl moiety, one substituent being always in para position, which  
5 substituent(s) is(are) selected from the group consisting of halogen, nitro, amino, alkyl (1-4 C), cyano, trifluoromethyl, hydroxy and alkoxy (1-4 C);  $R^3$  is D-Trp, L-Trp, L-Phe or L- or D-3-(2-naphtyl)-Ala;  $R^4$  is D-Lys, D-Arg,  
10 D-Orn, D-homo-Arg or D-His and  $R^5$  is Gly or D-Ala.

A preferred group of compounds of formula I is one in which X is hydrogen, lower alkanoyl,  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  wherein n is an integer from 2 to 6,  
15 or the acyl or N-alkanoyl-acyl portion of alanine, arginine, asparagine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenyl-alanine, proline, serine, threonine, tryptophan, tyrosine or valine;  
20  $R^1$  is D-3-(2-naphtyl)-Ala, L-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-Y-Phe wherein Y is selected from the group consisting of halo, nitro, amino, methyl, cyano, trifluoromethyl, hydroxy and methoxy;  
 $R^2$  is D-p-Y-Phe in which Y is as defined herein;  
25  $R^3$  is D-Trp;  $R^4$  is D-Lys or D-Arg and  $R^5$  is D-Ala or Gly.

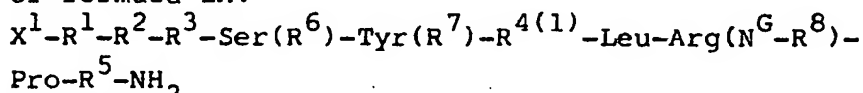
Another preferred group of the compounds of formula I is that in which X is hydrogen, lower alkanoyl or  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  wherein n is as  
30 defined herein;  $R^1$  is D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-halo-Phe;  $R^2$  is D-p-halo-Phe;  $R^3$  is D-Trp;  $R^4$  is D-Lys or D-Arg and  $R^5$  is D-Ala or Gly.

Still another preferred group of the compounds of formula I is that in which X is acetyl or  $\text{HOOCCH}_2\text{CH}_2\text{CO}$ ;  $\text{R}^1$  is D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-Cl-Phe;  $\text{R}^2$  is D-p-Cl-Phe;  
 5 D-p-Br-Phe or D-p-F-Phe;  $\text{R}^3$  is D-Trp;  $\text{R}^4$  is D-Lys or D-Arg and  $\text{R}^5$  is D-Ala.

The therapeutically acceptable salts of the compound of formula I are included within the scope of this invention.

10 The compounds of formula I are prepared by conventional peptide synthesis or by solid phase techniques.

In general, the peptides I may be prepared by removal of the protective group(s) from a peptide  
 15 of formula IA:

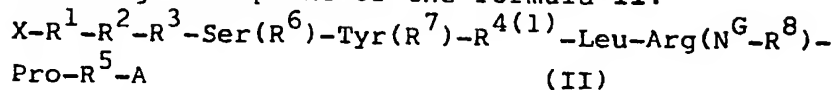


wherein  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and  $\text{R}^5$  are as herein defined,  $\text{X}^1$  has the same meaning as X as herein defined but

20 in addition may represent an N-protective group or an N-protected acyl portion of a D- or L-amino acid,  $\text{R}^{4(1)}$  has the same meanings as  $\text{R}^4$  defined already, but may in addition also represent  $\text{N}^E$ -protected D-lysyl,  $\text{N}^G$ -protected  
 25 D-ornithyl,  $\text{N}^G$ -protected D-arginyl,  $\text{N}^G$ -protected D-homo arginyl or N-protected histidyl, and  $\text{R}^6$ ,  $\text{R}^7$  and  $\text{R}^8$  are each hydrogen or a protective group, with the proviso that  
 30 at least one of the radicals  $\text{X}^1$ ,  $\text{R}^6$ ,  $\text{R}^7$ ,  $\text{R}^8$  or  $\text{R}^{4(1)}$  is or contains a protective group, and, if desired, followed by N-acylation of the resulting compound of formula I, wherein X is hydrogen.

The compounds of formula I may also be  
 35 prepared by a process, which comprises:

a) reacting a compound of the formula II:

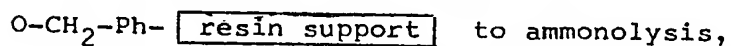


wherein

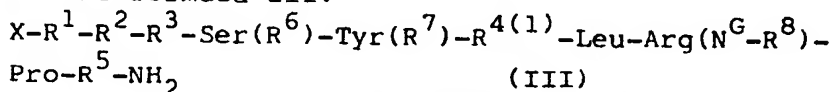
5 X, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4(1)</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>8</sup> are as defined herein and A is NHCHPh-Ph- Resin support with a reagent capable of cleaving off the resin support and the anchoring radical, i.e. -CHPh-Ph- resin support

10 or

b) subjecting the compound of the formula II wherein X, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4(1)</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>8</sup> are as defined herein and A is



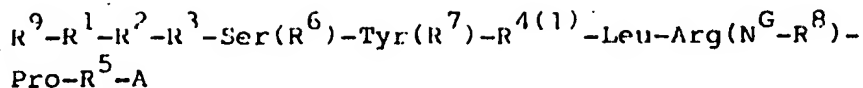
15 to obtain in both cases (a and b) a peptide of the formula III:



20 in which X, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4(1)</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>8</sup> are as defined herein; followed by, when the compound of formula III is different from the compound of formula I, reacting the compound of formula III with a reagent capable of removing the protecting groups without affecting the compound of formula I.

25 The compounds of formulae IA and II, and the compound of formula III when different from the compound of formula I, also are included within the scope of this invention as intermediates in the synthesis of the peptides of formula I.

30 A further aspect of the invention relates to intermediates linked to a solid resin support and having a protected  $\alpha$ -amino group. These intermediates are represented by the formula IV:



in which  $R^1$  to  $R^8$ , inclusive, are as defined herein and  $R^9$  is an  $\alpha$ -amino protective group

5 known to be useful in the art of the stepwise synthesis of polypeptides, suitable groups being listed hereinafter, and A is selected from the group consisting of NH-CHPh-Ph- resin support and OCH<sub>2</sub>-Ph- resin support

10

A gonadotropin antagonizing pharmaceutical composition is provided by admixing the compound of formula I with a pharmaceutically acceptable carrier.

15

Also provided is a method for relieving complications from the physiological availability of pituitary gonadotropins in a mammal, which involves administering to the mammal a gonadotropin antagonizing dose of the compound of formula I.

20

The term "alkanoyl" or "lower alkanoyl" as used herein means straight chain alkanoyl radicals containing one to six carbon atoms, e.g. formyl, acetyl, propionyl, butyryl and hexanoyl, and  
25 branched chained alkanoyl radicals containing four to six carbon atoms, e.g. isobutyryl and pivaloyl.

The term "lower alkanoic acid" as used herein means both straight and branched chain  
30 alkanoyl radicals containing two to six carbon atoms and includes acetic acid, propionic acid, pivalic acid, hexanoic acid and the like.

The term "organic proton acceptor" as used herein means the organic bases, or amines for  
35 instance, triethylamine, pyridine, N-ethyl-

morpholine, imidazole and the like.

The term "halo" or "halogen" includes chlorine, bromine, fluorine and iodine.

The term "amino acid" as used herein means  
5 the well known and reasonably accessible amino acids which are described in general textbooks on peptide chemistry; for instance, see K.D. Kopple, "Peptides and Amino Acids", W.A. Benjamin Inc., New York and Amsterdam,  
10 1966, pp. 4-7.

The term "acyl portion" of an amino acid means a radical derived from the corresponding amino acid by eliminating the hydroxyl of the carboxy group.

15 The term "amino acid residue" refers to a radical derived from the corresponding amino acid by eliminating the hydroxyl of the carboxy group and one hydrogen of the amino group.

$N^G$  means the side chain nitrogen atoms of  
20 arginine.

The symbol "Ph-" means phenyl and the symbol -Ph- means 1,4-phenylene.

"Ac" means acetyl. "Succ" means  $\text{HOOC}-(\text{CH}_2)_2-\text{CO}$ , i.e. succinyl, a 3-carboxy-1-propionyl radical.

25 "A" is an anchoring bond linked to a solid resin (resin support) used in solid phase synthesis and is selected from the class consisting of:  $-\text{NH}-\text{CHPh}-\text{Ph}-$  [resin support] and  $\text{OCH}_2-\text{Ph}-$  [resin support].

30 The term "anchoring radical" means that portion of A which is  $-\text{CH}-\text{Ph}-\text{Ph}-$  or  $-\text{CH}_2-\text{Ph}$ .

In general, the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-  
35 IUB Commission on Biochemical Nomenclature, see



Biochemistry II, 1726 (1972). For instance, t-Boc represents t-butyloxycarbonyl, Z represents benzyloxycarbonyl, Tos represents tosyl and Bzl represents benzyl. The abbreviations used herein  
5 for the various amino acids are Ala, alanine; Lys, lysyl, Arg, arginine, Gly, glycine; Leu, leucine; Orn, ornithyl, Phe, phenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; and Tyr, tyrosine. All amino acids described herein  
10 are in the L-series unless stated otherwise, e.g. D-Ala is a D-alanyl residue, D-Phe is a D-phenylalanyl residue and D-Trp is a D-tryptophyl residue.

The LH-RH analogs of this invention can be  
15 obtained in the form of an acid addition salt. Examples of salts are those with organic acids, e.g. acetic, lactic, succinic, benzoic, salicylic, methanesulphonic or p-toluenesulphonic acid, as well as polymeric acids, such as tannic  
20 acid or carboxymethyl cellulose, and salts with inorganic acids, such as hydrohalic acids, e.g. hydrochloric acid, or sulphuric acid, or phosphoric acid. If desired, a particular acid addition salt is converted into another acid  
25 addition salt, e.g. a salt with a non-toxic, therapeutically acceptable acid, by treatment with the appropriate ion exchange resin in the manner described by R.A. Boissonnas, et al., Helv.Chim.Acta., 43, 1349 (1960). Suitable ion  
30 exchange resins are cellulose based cation exchangers, for example carboxymethylcellulose or chemically modified, cross linked dextran cation exchangers, for example the chemically modified, cross-linked dextran cation exchanger  
35 sold under the trade mark Sephadex C, and

strongly basic anion exchange resins, for example those listed by J.P. Greenstein and M. Winitz in "Chemistry of the Amino Acids", John Wiley and Sons, Inc., New York and London, 1961, Vol. 2, p. 1456. These therapeutically acceptable acid addition salts are included within the scope of this invention.

The compounds of formula I in which X is  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  wherein n is an integer from 2 to 6 form salts with suitable therapeutically acceptable inorganic and organic bases. These derived salts possess the same activity as the parent acid and are included within the scope of this invention. The acid is transformed in excellent yield into the corresponding therapeutically acceptable salt by neutralization of said acid with the appropriate inorganic or organic base. Suitable inorganic bases to form these salts include, for example, the hydroxides, carbonates, bicarbonates or alkoxides of the therapeutically acceptable alkali metals or alkaline earth metals, for example, sodium, potassium, magnesium, calcium and the like. Suitable organic bases include the following amines; lower mono-, di- and trialkylamines, the alkyl radicals of which contain up to three carbon atoms, such as methylamine, dimethylamine, trimethylamine, ethylamine, di- and triethylamine, N-methyl-N-ethylamine, and the like; and mono-, di- and trialkanolamines, the alkanol radicals of which contain up to three carbon atoms, for example, mono-, di- and triethanolamine.

With reference to the compound of formula II,  
 $\text{X}-\text{R}^1-\text{R}^2-\text{R}^3-\text{Ser}(\text{R}^6)-\text{Tyr}(\text{R}^7)-\text{R}^{4(1)}-\text{Leu}-\text{Arg}(\text{N}^G-\text{R}^8)-$   
 $\text{Pro}-\text{R}^5-\text{A}$ , and the compound of formula III,

$X-R^1-R^2-R^3-Ser(R^6)-Tyr(R^7)-R^{4(1)}-Leu-Arg(N^G-R^8)-$   
 $Pro-R^5-NH_2$ , in a preferred embodiment X,  $R^1$ ,  $R^2$ ,  
 $R^3$ ,  $R^5$  and A are as defined herein,  $R^{4(1)}$  repre-  
sents D-Lys( $N^E-R^{10}$ ), D-Orn( $N^\delta-R^{10}$ ), D-Arg( $N^G-R^8$ ),  
5 D-homo Arg( $N^G-R^8$ ) or D-His( $N^I-R^{11}$ ),  $R^6$  is a  
protective group for the hydroxyl group of  
serine and is selected from the group of  
2-bromo-benzyloxy-carbonyl, benzyl, acetyl,  
tosyl, benzoyl, tert.butyl, tetrahydropyra-2-yl,  
10 trityl, 2,4-dichlorobenzyl and benzyloxycarbonyl;  
 $R^7$  is hydrogen or a protective group for the  
hydroxyl of tyrosine selected from the group  
defined hereinbefore for  $R^6$ ,  $R^8$  is a protective  
group for the  $N^\delta$ ,  $N^\omega$ , and  $N^\omega$  nitrogen atoms of  
15 arginine selected from the group consisting of  
tosyl, nitro, benzyloxycarbonyl and adamantyloxy-  
carbonyl,  $R^{10}$  is a protective group for the  
 $\epsilon$ -amino group of lysine or the  $\delta$ -amino group of or-  
nithine and is preferably selected from tosyl,  
20 tert.butyloxycarbonyl and 2-halo-benzyloxycarbonyl,  
and  $R^{11}$  is H or a protective group for the imidazol  
group of histidine and is preferably selected  
from benzyl, dinitrophenyl and trityl. In another  
preferred embodiment with reference to the  
25 compounds IA, II and III, the D-histidyl (if present),  
seryl and tyrosyl residues are not protected, the  
arginine residue(s) is(are) protected in the form  
of a strong acid addition salt, e.g., the hydro-  
chloric acid, p-toluenesulphonic acid or  
30 sulphuric acid addition salt, and the D-lysine or  
D-ornithine residues (if present) are protected  
by a 2-chloro or 2-bromo benzyloxycarbonyl group.

The valuable LH-RH antagonizing property of the compounds of this invention are demonstrated by standard pharmacological procedures. For example, this activity can be demonstrated in  
5 the test described by A. de la Cruz et al., Science, 191, 195 (1976). More explicitly, the assay is performed using mature female rats (Charles River Breeding Laboratories, Boston, Mass., U.S.A.) weighing about 200 g and  
10 exhibiting normal four-day cycles. The analogs are administered subcutaneously in 20% or 40% propylene glycol in physiologic saline or as a suspension in corn oil at 12 noon of the day of proestrus. On the following day, the rats are  
15 sacrificed, their fallopian tubes and uteri flushed with saline and the washes examined for ova.

The Tables I and II show the activity of  
20 some peptides of the invention, administered subcutaneously, in comparison with the reference peptide known from the U.S. patent specification No. 4,317,815.

25 Table I shows the anti-ovulatory activity using 40% propylene glycol in saline as the carrier substance. (the ref. peptide is in 20% propylene glycol in saline).

Table I

<u>Peptides</u>	dose in $\mu$ g s.c.	% blockade of ovulation
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-Phe <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH (reference peptide)	7.5 3	88 0
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-Lys <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	15 7.5 3	100 100 50
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-Arg <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	5 3	100 67
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-His <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	3	22
N-Ac-[D-3-(2-naphtyl)-Ala <sup>1</sup> , D-p-Cl-Phe <sup>2</sup> , D-Trp <sup>3</sup> , D-Arg <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	3 1	100 77

Table II shows the anti-ovulatory activity using a suspension of the peptide in Corn oil.

Table II

<u>Peptides</u>	dose in $\mu$ g s.c.	% blockade of ovulation
N-Ac-[D-Phe <sup>1,2</sup> , D-Trp <sup>3,6</sup> ] LH-RH (ref. Biochem. Biophys. Res. Commun. <u>86</u> , 1226, 1979)	250 100	100 40
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> D-Phe <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH (ref. copending pat. appln.)	3	0
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-Arg <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	3 1.5 0.750	100 90 40
N-Ac-[D-p-Cl-Phe <sup>1,2</sup> , D-Trp <sup>3</sup> , D-Lys <sup>6</sup> , D-Ala <sup>10</sup> ]-LH-RH	3	70

In contrast to prior art peptides the present peptides possess also surprisingly potent oral activity. In order to investigate the oral anti-ovulatory activity, rats were starved  
5 for 24 hours prior to the administration of the peptide to be tested. The peptide is dissolved or emulgated in a suitable vehicle; one ml solution or emulsion was given by stomach tube.

10 Table III

Anti-ovulatory activity of the reference peptide  
N-Ac-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Phe<sup>6</sup>-D-Ala<sup>10</sup>]-  
LH-RH administered by gavage: 2 mg/ml/rat at  
9.30 AM of pro-estrus

<u>Vehicle</u>	<u>% inhibition of ovulation</u>
40% propylene glycol in lipomul oral and 1000 Int.Units trasyolol	0
40% propylene glycol in coconut oil and 1000 Int.Units trasyolol	0

Anti-ovulatory activity of the peptide of this invention:

N-Ac-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-

LH-RH administered by gavage: 2 mg/ml/rat at

5 2 PM of pro-estrus

Vehicle	% inhibition of ovulation
40% propylene glycol in saline and 1000 Int.Units of trasyolol	100
40% propylene glycol in coconut oil and 1000 Int.Units of trasyolol	100

The LH-RH antagonizing properties of the peptides of this invention make the compounds useful in human and veterinary practice. For instance, the compounds of formula I find use as agents for relieving the complications from the undesirable physiological availability of pituitary gonadotropins in a mammal. Such complications include precocious puberty; hormone dependent tumors such as malignant and benign prostatic tumors, and mammary, ovarian and testicular tumors; hirsutism; acne; amenorrhea, e.g. secondary amenorrhea; endometriosis, and ovarian and mammary cystic diseases in both animals and humans. The compounds of formula I also are useful for regulating ovulation, thus rendering them useful agents for controlling fertility, e.g. as precoital or postcoital contraceptives, for synchronizing estrus in livestock and for improving the "rhythm" method. Also, the compounds are useful



for regulating the human menopausal gonadotropin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) during perimenopausal and postmenopausal periods in women.

5       When the compound of formula I, preferably in the form of an acid addition salt, is employed in human or veterinary medicine, it is administered systemically, either orally or by subcutaneous or intramuscular injection, or  
10 by sublingual, nasal, or vaginal administration, in compositions in conjunction with a pharmaceutically acceptable vehicle or carrier.

      The daily dosage of the compounds of formula I will vary with the form of administration  
15 and with the particular patient under treatment. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum  
20 effect under the circumstances is reached. In general, the compound obtained by the process is most desirably administered at a concentration level that generally will inhibit release of LH and of FSH without causing any harmful or  
25 deleterious side effects, and preferably at a level that is in a range of from 1 mcg to about 1000 mcg per kilogram bodyweight for parenteral administration and from 0.5 mg to about 100 mg per kilogram bodyweight for enteral administration.  
30 However, a dosage level for parenteral administration that is in the range of from 2.5 mcg to about 250 mcg and for enteral administration of from 1 mg to 25 mg is most desirably employed in order to achieve effective  
35 results.

For administration by the nasal route as drops or spray it is preferred to use the compound of formula I in solution in a sterile aqueous vehicle which may also contain other  
5 solutes such as buffers or preservatives, as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic. Doses by the intranasal route range from 100 mcg to 10.0 mg/kg, or preferably  
10 100 mcg to 1.0 mg/kg of body weight.

The compound of formula I also may be administered as nasal or vaginal powders or insufflations. For such purposes the decapeptide is administered in finely divided solid form  
15 together with a pharmaceutically acceptable solid carrier; for example, a finely divided polyethylene glycol for instance the polyethylene glycol sold under the trade mark "Carbowax 1540"; finely divided lactose; or preferably for vaginal  
20 administration, very finely divided silica, for instance, the silica sold under the trade mark "Cab-O-Sil". Such compositions may also contain other excipients in finely divided solid form such as preservatives, buffers, or surface  
25 active agents.

For sublingual or vaginal administration, the compound is formulated preferably in solid dosage forms such as sublingual tablets or vaginal inserts or suppositories with sufficient  
30 quantities of solid excipients such as starch, lactose, certain types of clay, buffers, and lubricating, disintegrating, or surface-active agents, or with semi solid excipients commonly used in the formulation of suppositories.  
35 Examples of such excipients are found in standard

pharmaceutical texts, e.g. in Remington's  
Pharmaceutical Sciences, Mack Publishing  
Company, Easton, Pa., 1970.

It is often desirable to administer the  
5 compounds of formula I continuously over  
prolonged periods of time in long-acting,  
slow-release, or depot dosage forms. Such  
dosage forms may either contain a pharmaceuti-  
cally acceptable salt of the compound having a  
10 low degree of solubility in body fluids, for  
example one of those salts described below, or  
they may contain the compound in the form of a  
water-soluble salt together with a protective  
carrier which prevents rapid release. In the  
15 latter case, for example, the compound may be  
formulated with a non-antigenic partially  
hydrolyzed gelatin in the form of a viscous  
liquid; or it may be adsorbed on a pharmaceuti-  
cally acceptable solid carrier, for example,  
20 zinc hydroxide, and may be administered in  
suspension in a pharmaceutically acceptable  
liquid vehicle; or the decapeptide may be  
formulated in gels or suspensions with a  
protective non-antigenic hydrocolloid, for  
25 example sodium carboxymethylcellulose, polyvinyl-  
pyrrolidone, sodium alginate, gelatine, poly-  
galacturonic acids, for example, pectin, or  
certain mucopolysaccharides, together with  
aqueous or non-aqueous pharmaceutically  
30 acceptable liquid vehicles, preservatives, or  
surfactants. Examples of such formulations are  
found in standard pharmaceutical texts, e.g. in  
Remington's Pharmaceutical Sciences, cited above.  
Long-acting, slow-release preparations of the  
35 compound may also be obtained by micro-

encapsulation in a pharmaceutically acceptable coating material, for example gelatine, polyvinyl alcohol or ethyl cellulose. Further examples of coating materials and of the processes used for microencapsulation are described by J.A. Herbig in "Encyclopedia of Chemical Technology", Vol.13, 2nd ed., Wiley, New York, 1967, pp. 436-456. Such formulations, as well as suspensions of salts of the compound which are only sparingly soluble in body fluids, are designed to release from about 1-1000 mcg of the compound per kilogram body weight per day, and are preferably administered by intramuscular injection. Alternatively, some of the solid dosage forms listed above, for example certain sparingly water-soluble salts or dispersions in or adsorbates on solid carriers of salts of the compound, for example dispersions in a neutral hydrogel of a polymer of ethylene glycol methacrylate or similar monomers, cross-linked as described in U.S. Patent 3,551,556, issued December 29, 1970 to K. Kliment, et al., may also be formulated in the form of pellets releasing about the same amounts as shown above and may be implanted subcutaneously or intramuscularly.

Alternatively, slow-release effects over prolonged periods of time may also be obtained by administering the compound obtained by the process of this invention in an intra-vaginal device or in a temporary implant, for example a container made of a non-irritating silicone polymer such as polysiloxane, a suitable polysiloxane is sold under the trade mark "Silastic", or of a neutral hydrogel of a polymer as described above, possessing the required degree of

permcability to release from about 0.1 mcg to about 50 mcg per kilogram body weight per day. Such intravaginal or implant dosage forms for prolonged administration have the advantage that  
5 they may be removed when it is desired to interrupt or to terminate treatment.

#### Chemical synthesis

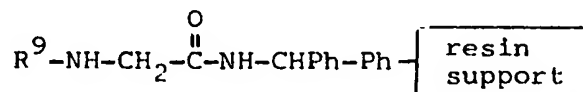
In selecting a particular side chain protective group to be used in the synthesis of  
10 the present decapeptides, the following rules should be followed:

- (a) the protective group must be stable to the reagent and under the reaction conditions selected for removing the  $\alpha$ -amino protective  
15 group at each step of the synthesis,
- (b) the protective group must retain its protecting properties (i.e., not be split off under coupling conditions), and
- (c) the side chain protective group must be  
20 removable upon the completion of the synthesis of the peptide containing the desired amino acid sequence under reaction conditions that will not alter the peptide chain.

With reference to  $\alpha$ -amino protective groups,  
25 for instance  $R^9$ , suitable protective groups include (1) aliphatic urethan protective groups illustrated by t-butyloxycarbonyl, diisopropylmethoxycarbonyl, biphenylisopropyloxycarbonyl, isopropyloxycarbonyl, t-amylloxycarbonyl, ethoxy-  
30 carbonyl and allyloxycarbonyl; (2) cycloalkyl urethan type protective groups illustrated by cyclopentyloxycarbonyl, adamantyloxycarbonyl, d-isobornyloxycarbonyl and cyclohexyloxycarbonyl; and nitrophenylsulfenyl;  $\alpha, \alpha$ -dimethyl-3,5-dimethoxy-  
35 benzyloxycarbonyl; and trityl. The preferred

$\alpha$ -amino protective groups are selected from the group consisting of t-butyloxycarbonyl, cyclopentylloxycarbonyl, t-amylloxycarbonyl, d-isobornylloxycarbonyl, o-nitrophenylsulfenyl, biphenylisopropylloxycarbonyl, and  $\alpha,\alpha$ -dimethyl-3,5-dimethoxybenzylloxycarbonyl.

Using solid phase techniques the synthesis is commenced from the C-terminal end of the peptide using an  $\alpha$ -amino protected amino acid linked to a solid resin. Such a starting material is prepared for example, by attaching an  $\alpha$ -amino protected glycine to a benzhydrylamine resin, a chloromethylated resin or a hydroxymethyl resin, the former being preferred. The preparation of a benzhydrylamine resin is described by P. Rivaille, et al., *Helv.Chim.Acta.*, 54, 2772 (1971) and the preparation of the hydroxymethyl resin is described by M. Bodanszky and J.T. Sheehan, *Chem.Ind. (London)*, 38, 1597 (1966). A chloromethylated resin is commercially available from Bio Rad Laboratories, Richmond, California. In using the benzhydrylamine resin, an amide anchoring bond is formed with the  $\alpha$ -amino protected glycine or D-alanine, illustrated as follows for glycine:



This permits the C-terminal amide function to be obtained directly after the amino acid sequence in the synthesis is completed by cleaving off the resin support and anchoring radical of the linked peptide to form the amino acid amide at the C-terminal portion of the desired compound. In this instance the use of

hydrogen fluoride for cleaving off the resin support also removes the side chain protective groups to give the decapeptide of this invention.

When the other resins are used, the

- 5 anchoring bond is the benzylester group as illustrated hereinbefore. In this instance a convenient procedure for converting the linked protected peptide to the C-terminal amide is to ammonolize the protected peptide off the resin  
10 and then remove the protective groups of the resulting amide by treatment with sodium in liquid ammonia or by the hydrogen fluoride cleavage. An alternative procedure would be to cleave by transesterification with a lower  
15 alkanol, preferably methanol or ethanol, in the presence of triethylamine and then convert the resulting ester into an amide and subsequently deprotect as described above. See also J.M. Steward and J.D. Young, "Solid Phase  
20 Peptide Synthesis", W.H. Freeman & Co., San Francisco, 1969, pp. 40-49.

- More specifically, in an embodiment of the present invention, an  $\alpha$ -amino protected amino acid; namely, an  $\alpha$ -amino protected glycine,  
25 preferably t-butyloxycarbonylglycine; or an  $\alpha$ -amino protected D-alanine, preferably t-butyl-oxycarbonyl-D-alanine; is coupled to the benz-hydrylamine resin with the aid of a carboxy group activating compound, preferably, dicyclohexyl-  
30 carbodiimide or diisopropylcarbodiimide. Following the coupling of the  $\alpha$ -amino protected amino acid to the resin support, the  $\alpha$ -amino protecting group is removed such as by using trifluoroacetic acid in methylene chloride,  
35 trifluoroacetic acid alone or hydrochloric acid

in dioxane. The deprotection is carried out at a temperature between 0 °C and room temperature (i.e. 20 to 24 °C). Other standard cleaving reagents and conditions for removal of specific

5  $\alpha$ -amino protecting groups may be used as described by E. Schroder and K. Lubke, "The Peptides", Vol. I, Academic Press, New York, 1965, pp. 72-75. After removal of the  $\alpha$ -amino protecting group, the remaining  $\alpha$ -amino protected

10 amino acids are coupled step-wise in the desired order to obtain the peptide. Each protected amino acid is introduced into the solid phase reactor in about a three-fold excess and the coupling is carried out in a medium of methylene chloride or

15 mixtures of dimethyl-formamide in methylene chloride. In cases where incomplete coupling occurred, the coupling procedure is repeated before removal of the  $\alpha$ -amino protecting group, prior to the coupling of the next amino acid to the solid

20 phase reactor. The success of the coupling reaction at each stage of the synthesis is monitored by the ninhydrin reaction as described by E. Kaiser, et al., *Analyt. Biochem.*, 34, 595 (1970). In this manner the compounds of formula II in which X is hydrogen

25 is obtained.

After the desired amino acid sequence has been synthesized, the peptide is removed from the resin support by treatment with a reagent such as hydrogen fluoride which not only cleaves the

30 peptide from the resin but also cleaves all remaining side chain protecting groups to obtain directly the peptide of formula I wherein X is hydrogen in the case where the benzhydrylamine resin was used.



Where a benzylester resin is used the peptide may be separated from the resin by transesterification with a lower alkanol, preferably methanol or ethanol, after which the recovered  
5 product is chromatographed on silica gel and the collected fraction subjected to treatment with ammonia to convert the lower alkyl ester, preferably the methyl or ethyl ester, to the C-terminal amide. In this manner the compounds  
10 of formula III in which X is hydrogen are obtained. The side chain protecting groups are then cleaved by procedures described above, for example by treatment with sodium in liquid ammonia or by hydrogen fluoride to give the corresponding  
15 compound of formula I wherein X is hydrogen.

The peptide compounds of formula I in which X is other than hydrogen are preferably obtained by acylation of the corresponding compound of formula II or III in which X is  
20 hydrogen with the appropriate acylating agent, followed by cleaving the resin support together with the anchoring radical, and all the remaining side chain protecting groups of the acylated product by the procedures described above.  
25 Preferably, a lower alkanolic acid chloride or bromide or lower alkanolic anhydride in the presence of an organic proton acceptor, e.g. pyridine or imidazole, is used as the acylating agent to prepare the compounds of formula II or  
30 III in which X is lower alkanoyl. Likewise, in the presence of an organic proton acceptor, compounds of formula II or III in which X is  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  are prepared using the acid chloride or acid bromide corresponding to the  
35 desired acyl radical X, e.g. succinyl chloride

can be used for preparing compounds of formula II or III in which X is  $\text{HOOCCH}_2\text{CH}_2\text{CO}$ . The compound of formula II or III in which X is the acyl portion or N-acylated acyl portion of a D- or L-amino acid are prepared conveniently by the "activated ester" coupling procedure. Accordingly, the amino acid corresponding to the desired acyl portion to be incorporated into the compound of formula II or III is converted into an activated ester. Descriptions of such carboxyl-activating groups are found in general textbooks of peptide chemistry; for example K.D. Kopple, "Peptides and Amino Acids", W.A. Benjamin, Inc., New York, 1966, pp. 45-51, and E. Schröder and K. Lübke, "The Peptides"; Vol. I, Academic Press, New York, 1965, pp. 77-128. Examples of the activated form of the terminal carboxyl are acid chloride, anhydride, azide, activated ester, or O-acyl urea of a dialkylcarbodiimide. The following activated esters have proved to be particularly suitable in the process of this invention: 2,4,5-trichlorophenyl (represented by TcP), pentachlorophenyl (represented by Pep), p-nitrophenyl (represented by Np); the 1-benzotriazolyl group (represented by Bt) or the succinimido group is also useful for such activation.

The peptide compounds of formula I in which X is hydrogen also are useful for preparing the compounds of formula I in which X is other than hydrogen by subjecting the former compound to the appropriate acylating agent.

Although a solid phase synthesis of the compound of formula I is disclosed herein in detail, the preparation of the peptide also can be realized by classical solution methods well-

known and described in detail in all textbooks, dealing with peptide chemistry.

Preparation of the resins

5 By way of example the preparation of some resins are described hereunder.

A. D-p-chlorophenylalanyl-D-p-chlorophenyl-  
alanyl-D-tryptophyl-L-seryl (O-benzyl)-L-  
10 tyrosyl-D-arginyl (N<sup>G</sup>-tosyl)-L-leucyl-L-  
arginyl (N<sup>G</sup>-tosyl)-L-prolyl-D-alanyl-benz-  
hydramine resin;

Benzhydramine resin (1.05 g, 0.5 mmole)  
15 was placed in the reaction vessel of a Beckman Model 990 automatic peptide synthesizer programmed to carry out the following work-wash cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1  
20 and 25 min each); (c) methylenechloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform (2 times for 3 min. each); (g) methylene chloride.

The washed resin was stirred with t-butyl-  
25 oxycarbonyl D-alanine (320 mg, 1.5 mmoles) in methylene chloride and diisopropylcarbodiimide (1.5 mmoles) was added. The mixture was stirred at room temperature for 1 h. and the amino acid resin was then washed with methylene chloride.  
30 The protected, attached amino acid was then cycled through steps (b) through (g) in the above wash program. The following amino acids (1.5 mmoles) were then coupled successively by the same cycle of events: t-Boc-proline, t-Boc-L-arginine  
35 (N<sup>G</sup>-Tos), t-Boc-L-leucine; t-Boc-D-arginine (N<sup>G</sup>-Tos), t-Boc-L-tyrosine, t-Boc-L-serine (O-Bzl),

t-Boc-D-tryptophan, t-Boc-D-p-chlorophenylalanine  
and t-Boc-D-p-chloro-phenylalanine.

Upon completion of the last coupling reaction  
the N-terminal t-Boc group was removed whereupon  
5 the resulting resin was washed with methanol and  
dried.

B. N-acetyl-D-p-chlorophenylalanyl-D-p-chloro-  
phenylalanyl-D-tryptophyl-L-seryl (O-benzyl)-L-  
10 tyrosyl-D-arginyl ( $N^G$ -tosyl)-L-leucyl-L-  
arginyl ( $N^G$ -tosyl)-L-prolyl-D-alanyl-benz-  
hydramine resin;

The resin obtained in A. was treated for  
15 30 min. with 60 ml of a solution of imidazole  
(5 g) and acetic anhydride (3.54 ml) in methylene  
chloride (100 ml). The acetylated peptide-resin  
was then washed with methylene chloride and  
methanol and dried.

20

C. D-p-chlorophenylalanyl-D-p-chlorophenylalanyl-  
D-tryptophyl-L-seryl (O-benzyl)-L-tyrosyl-D-  
lysyl ( $N^E$ -2-chlorobenzoyloxycarbonyl)-L-leucyl-  
L-arginyl ( $N^G$ -tosyl)-L-prolyl-D-alanyl-benz-  
25 hydramine resin.

The resin was manufactured on the same  
scale and under the same conditions as described  
in A. with the exception that t-Boc-D-lysine  
30 ( $N^E$ -2-chlorobenzoyloxycarbonyl) was incorporated  
in place of t-Boc-D-Arginine ( $N^G$ -Tosyl).

D. The corresponding N-terminal acetyl derivative  
of the resin obtained in C. was prepared in the  
35 same manner as described in B.

E. D-3-(2-naphtyl)-alanyl-D-p-chlorophenyl-  
alanyl-D-tryptophyl-L-seryl-(O-benzyl)-L-  
tyrosyl-D-Arginyl ( $N^G$ -tosyl)-L-leucyl-L-  
arginy ( $N^G$ -tyrosyl)-L-prolyl-D-alanyl-benz-  
5 hydrylamine resin.

The resin was manufactured on the same scale  
and under the same conditions as described in A  
with the exception that as the N-terminal amino  
10 acid was coupled t-Boc-D-3-(2-naphtyl)-alanine in  
place of t-Boc-D-p-chloro-phenyl-alanine.

F. N-acetyl-D-3-(2-naphtyl)-alanyl-D-p-chloro-  
phenylalanyl-D-tryptophyl-L-seryl-(O-benzyl)-  
15 L-tyrosyl-D-arginy ( $N^G$ -tosyl)-L-leucyl-L-  
arginy ( $N^G$ -tyrosyl)-L-prolyl-D-alanyl-benz-  
hydrylamine resin.

The resin obtained in E. was acetylated as  
20 described in B. The acetylated peptide resin was  
then washed with methylenechloride and methanol  
and dried.

G. L-3-(2-naphtyl)-alanyl-D-p-chlorophenyl-  
25 alanyl-D-tryptophyl-L-seryl-(O-benzyl)-L-  
tyrosyl-D-lysyl ( $N^E$ -2-chlorobenzyloxycarbonyl)-  
L-leucyl-L-arginy ( $N^G$ -tosyl)-L-prolyl-D-  
alanyl-benzhydrylamine resin.

The resin was manufactured on the same scale and  
30 under the same conditions as described in E. with the  
exception that t-Boc-D-lysine( $N^E$ -2-chlorobenzyloxy-  
carbonyl) was incorporated in place of t-Boc-D-  
Arginine( $N^G$ -tosyl) and L-3-(2-naphtyl)-alanine  
instead of D-3-(2-naphtyl)-alanine.

35

H. The corresponding N-terminal acetyl derivative of the resin obtained in G was prepared in the same manner as described in B.

- 5 K. The corresponding N-terminal-succinyl derivative of the resin described in A was obtained by reacting 0.75 g of the resin A with 30 ml of a solution of imidazol and succinylchloride in methylenechloride for 30 minutes. The acylated  
10 peptide-resin was then washed with methylenechloride and methanol and dried in vacuo.

The following examples illustrate further this invention. In the examples, the ratio noted  
15 in connection with a mixture of solvents refers to the relative proportion of the solvents with respect to volume.

Example 1

20 N-Ac-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH

Removal of the protecting groups (with the exception of the N-acetyl group) and cleavage of the decapeptide from the resin described previously (see A. and B.) was carried out by treatment of  
25 1.96 g of the resin with hydrogen fluoride (40 ml), anisole (10 ml), and 1,4-dithiothreitol (100 mg) at 0 °C for 1 h. The hydrogen fluoride was removed under a nitrogen stream and the peptide precipitated by addition of ether.

30 The crude peptide was extracted with 50% acetic acid. The extract was subjected to gel filtration on a column (2.5 x 95 cm) of a fine grade chemically modified cross-linked dextran, sold under the trade mark "Sephadex G-25", using  
35 50% acetic acid as the solvent. Fractions shown

to contain a major peak by UV absorption at 280 nm were pooled and evaporated to dryness. The residual oil was applied to a column (2.5 x 45 cm) of octadecylsilane bonded silica gel and eluted with a linear gradient of 10% to 40% n-propanol in 20% acetic acid 0.1 M ammonium acetate. The major peak was collected, evaporated to an oil and lyophilized in dilute acetic acid to give the title compound (232 mg) as a white powder. The product was homogeneous when examined by thin layer chromatography on silica gel in 4 different solvent systems when loads of 20-30 mcg were applied and spots visualized by  $\text{Cl}_2$ -starch reagent and Ehrlich reagent. The following  $R_f$  values were obtained: (A) 1-butanol: acetic acid: water (4:1:1), 0.43; (B) ethyl acetate:pyridine:acetic acid:water (20:5:1:3), 0.12; (C) 2-propanol:1 M acetic acid (2:1), 0.43; (D) 1-butanol:acetic acid:water:ethyl acetate (1:1:1:1), 0.64. Amino acid analysis gave: Ser, 0.80; Pro, 0.97; Ala, 1.00; Leu, 1.03; Tyr, 1.00; p-Cl-Phe, 2.00; Trp, 0.66; Arg, 1.81.

#### Example 2

N-Ac-[D-3-(2-naphtyl)-Ala<sup>1</sup>, D-p-Cl-Phe<sup>2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH

The title compound was prepared from the resin described under E or F in the way described in Example 1.

The crude peptide was purified using the same extraction and chromatographic methods as described in Example 1.

Example 3

N-Ac-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Lys<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH

Removal of the protecting groups (with the exception of the N-acetyl group and cleavage of the decapeptide from the resin (see D.) was accomplished under the same conditions as described in Example 1.

The crude peptide was purified using the same extraction and chromatographic methods as described in Example 1.

Example 4

N-Ac-[D-3-(2-naphtyl)-Ala<sup>1</sup>, D-p-Cl-Phe<sup>2</sup>, D-Trp<sup>3</sup>, D-Lys<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH

Removal of the protecting groups (with the exception of the N-acetyl group and cleavage of the decapeptide from the resin (see H.) was accomplished under the same conditions as described in Example 1.

The crude peptide was purified using the same extraction and chromatographic methods as described in Example 1.

Example 5

In the same manner as described in Example 1 the following peptides were prepared:

[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH,

[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Lys<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH,

N-Suc-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH,

N-Ac-[D-p-Cl-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-His<sup>6</sup>, D-Ala<sup>10</sup>]-LH-RH,



- N-Ac-[D-p-Cl-Phe<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>]-LH-RH,
- N-Ac-Gly-[D-p-Cl-Phe<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- 5 N-Ac-[D-Phe<sup>1</sup>,D-p-F-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- N-Ac-[D-p-Cl-Phe<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Orn<sup>6</sup>-D-Ala<sup>10</sup>]-LH-RH,
- 10 N-Ac-[D-p-Cl-Phe<sup>1,2</sup>,D-β-naphtyl-Ala<sup>3</sup>,D-Lys<sup>6</sup>-D-Ala<sup>10</sup>]-LH-RH,
- N-Ac-[D-Trp<sup>1</sup>,D-p-Cl-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- 15 N-Ac-[ -p-Cl-Phe<sup>1,2</sup>,Phe<sup>3</sup>,D-homo-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- [D-3-(2-naphtyl)-Ala<sup>1</sup>,D-p-Cl-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- 20 [D-3-(2-naphtyl)-Ala<sup>1</sup>,D-p-Cl-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Lys<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- [L-3-(2-naphtyl)-Ala<sup>1</sup>,D-p-Cl-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- 25 N-Ac-[L-3-(2-naphtyl)-Ala<sup>1</sup>,D-p-F-Phe<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH,
- N-Ac-[D-p-Cl-Phe<sup>2</sup>,D-3-(2-naphtyl)-Ala<sup>1,3</sup>,D-Lys<sup>6</sup>,D-Ala<sup>10</sup>]-LH-RH.
- 30

CLAIMS

1. A peptide of formula I  
$$X-R^1-R^2-R^3-Ser-Tyr-R^4-Leu-Arg-Pro-R^5-NH_2 \quad (I)$$
in which X is hydrogen, lower alkanoyl,  $HOOC-(CH_2)_n-CO$  wherein n is an integer from 2 to 6, or the acyl or N-alkanoyl-acyl portion of glycine or a D- or L-amino acid;  $R^1$  is Gly, L-Ala, L-3-(1-naphtyl)-Ala, L-3-(2-naphtyl)-Ala, D-Ala, D-3-(1-naphtyl)-Ala, D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-Phe having one or more substituents at the phenyl moiety selected from the group consisting of halogen, nitro, amino, alkyl (1-4 C), cyano, trifluoromethyl, hydroxy and alkoxy (1-4 C);  $R^2$  is D-Phe having one or two substituents at the phenyl moiety, one substituent being always in para position which substituent(s) is(are) selected from the group consisting of halogen, nitro, amino, alkyl (1-4 C), cyano, trifluoromethyl, hydroxy and alkoxy (1-4 C);  $R^3$  is D-Trp, L-Trp, L-Phe or L- or D-3-(2-naphtyl)-Ala;  $R^4$  is D-Lys, D-Arg, D-Orn, D-homo-Arg or D-His and  $R^5$  is Gly or D-Ala; or a therapeutically acceptable salt thereof.

2. The compound of Claim 1 in which X is hydrogen, lower alkanoyl,  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  wherein n is an integer from 2 to 6, or the acyl or N-alkanoyl-acyl portion of an amino acid residue of alanine, arginine, asparagine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine;  $\text{R}^1$  is D-3-(2-naphtyl)-Ala, L-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-Y-Phe wherein Y is selected from the group consisting of halogen, nitro, amino, methyl, cyano, trifluoromethyl, hydroxy and methoxy;  $\text{R}^2$  is D-p-Y-Phe in which X is as defined herein;  $\text{R}^3$  is D-Trp;  $\text{R}^4$  is D-Lys or D-Arg and  $\text{R}^5$  is Gly or D-Ala; or a therapeutically acceptable salt thereof.
3. The compound of Claim 1 in which X is hydrogen, lower alkanoyl or  $\text{HOOC}-(\text{CH}_2)_n-\text{CO}$  wherein n is an integer from 2 to 6;  $\text{R}^1$  is D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-halo-Phe;  $\text{R}^2$  is D-p-halo-Phe;  $\text{R}^3$  is D-Trp;  $\text{R}^4$  is D-Lys or D-Arg and  $\text{R}^5$  is Gly or D-Ala; or a therapeutically acceptable salt thereof.
4. The compound of Claim 1 in which X is acetyl or  $\text{HOOCCH}_2-\text{CH}_2\text{CO}$ ,  $\text{R}^1$  is D-3-(2-naphtyl)-Ala, D-Trp, D-Phe or D-p-Cl-Phe;  $\text{R}^2$  is D-p-Cl-Phe; D-p-Br-Phe or D-p-F-Phe;  $\text{R}^3$  is D-Trp;  $\text{R}^4$  is D-Lys or D-Arg and  $\text{R}^5$  is D-Ala; or a therapeutically acceptable salt thereof.

5. D-p-chloro-Phenylalanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-propyl-D-alaninamide, as claimed in Claim 1.
6. N-Acetyl-D-p-chlorophenylalanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
7. D-p-chloro-Phenylalanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
8. N-Acetyl-D-p-chlorophenylalanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
9. N-Succinyl-D-p-chloro-phenylalanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
10. N-Acetyl-D-p-chlorophenylalanyl-D-p-chlorophenylalanyl-D-tryptophyl-L-seryl-L-tyrosyl-D-histidyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
11. N-Acetyl-D-tryptophyl-D-p-chlorophenylalanyl-D-tryptophyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.

12. N-Acetyl-D-phenylalanyl-D-p-chlorophenylalanyl-D-tryptophyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
13. D-3-(2-naphtyl)-alanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
14. N-Acetyl-D-3-(2-naphtyl)-alanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-arginyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
15. D-3-(2-naphtyl)-alanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
16. N-Acetyl-D-3-(2-naphtyl)-alanyl-D-p-chlorophenylalanyl-D-tryptophanyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, as claimed in Claim 1.
17. A gonadotropin antagonizing pharmaceutical composition comprising a gonadotropin antagonizing amount of the compound of Claim 1 or a therapeutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
18. A gonadotropin antagonizing oral pharmaceutical composition comprising a gonadotropin antagonizing amount of the compound of Claim 1 or a therapeutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

19. A compound of the formula II  
 $X-R^1-R^2-R^3-Ser(R^6)-Tyr(R^7)-R^{4(1)}-Leu-Arg(N^G-R^8)-$   
 $Pro-R^5-A$

wherein

$X, R^1, R^2, R^3$  and  $R^5$  have the meanings defined  
 in claim 1,

$R^{4(1)}$  has the same meanings as  $R^4$  defined in  
 claim 1 but in addition may also represent  
 $N^E$ -protected D-Lys,  $N^{\omega}$  protected D-Orn,  $N^G$  pro-  
 tected D-Arg,  $N^G$  protected D-homo-Arg or  $N^I$  pro-  
 tected His,

$R^6, R^7$  and  $R^8$  are each hydrogen or a protective  
 group and

A represents  $-NH-CH-Ph-Phe-$  resin  
support or

$-OCH_2-Ph-$  resin  
support .



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# EUROPEAN SEARCH REPORT

0081877  
Application number

EP 82 20 1544

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
A	--- Chemical Abstracts vol. 94, no. 5, 2 February 1981, Columbus, Ohio, USA, D.H. COY et al. "LH-RH antagonists with potent antitumor activity", abstract no. 25416u & Pept. Struct. Biol. Funct. Proc. Am. Pept. Symp. 6th, 1979, pages 775-779	1-19	C 07 C 103/52 A 61 K 37/02
A	--- EP-A-0 041 286 (COY) * Examples * & GB-A-2 053 229 -----	1-19	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 3)
			C 07 C 103/52 A 61 K 37/02
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 03-02-1983	Examiner BREW C.H.
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